Expt: PMC9 amplification with 2 U of enzyme + different amounts of Deep Vent.

Repeat of previous expt, 4 of points here.

200 μM dNTP
0.4 μM primers
50 pg Template
2 mM Mg
2 U Taq

	D.V.						
	1,	0.5,	0.2,	0.1,	0.05,	0.02,	0.01,
	0.005,	0.002,	0.001,	0			

U/λ diluted to 0.1 U/λ → 1/10 = 0.01 U/λ → 1/10 = 0.001 U/λ
in 1x buffer w/o Mg.

Prepared premix 25x, done in duplicate.
45 μl of " + 5 μl of different amounts of enzyme.

	H_2O
10x buffer	125 μl
dNTP 10 mM	25
Mg 100 mM	25
primer 1	10.6
2	9.5
λ Template	25.0
	1125 ⇐ added 2.5 μl Taq = 25 U

removed 40 μl = w/o any enzyme

After adding Taq, mixed & aliquoted 45 μl / 2x to dif. Tubes.

added Deep Vent diluted different conc.

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Read & Understood by me,	Date	Invented by	Date
	1/9/95	Recorded by K. Sivaraman	12/27/94

[redacted]

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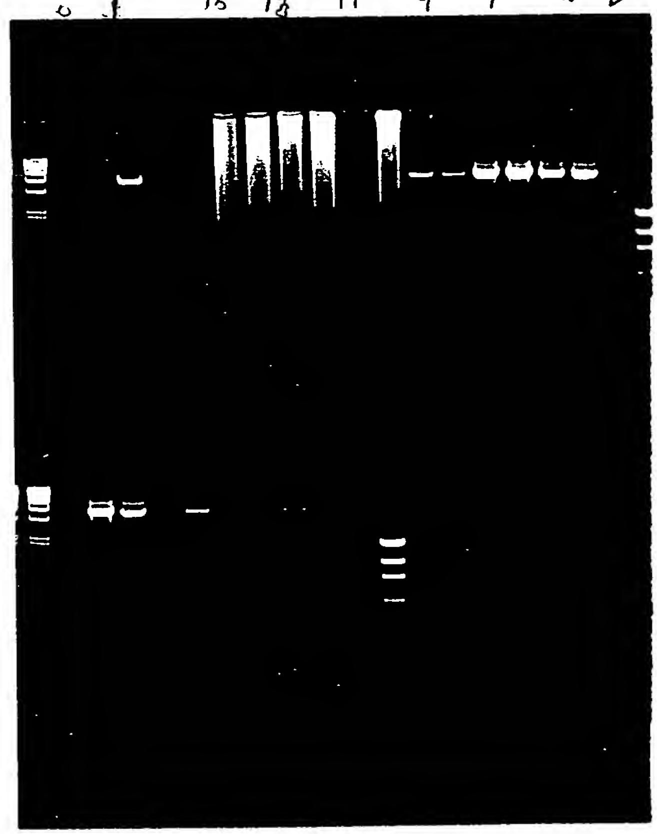
					11,20
0	–	no enzyme			
1	–	4U Taq +	0.02U DV		
2	–	2U Taq	0 DV		0 + 5
3 4	–	↓	.01	} 0.01U/λ	1 + 4
5 6			.02		2 + 3
7 8			.05		5 + 0
9 10			.1	} 0.1U/λ	1 + 4
11 12			.2		2 + 3
13 14			.5	} original	0.25 + 4.75
15 16		↓	1		0.5 + 4.5
17 18		2U Taq	0.005	} 0.001U/λ	5 + 0
19 20		↓	0.002		2 + 3
21 22			0.001		1 + 4

23 2U Taq alone = 2

Cycling: 94°, 30″

35 (94°, 30″, 5′, 68°)



Result:

2U Taq + 0.02U DV works real good. In last expt didn't work.

2U Taq alone between 1 & 15 in picture is very faint.

2U Taq + even .001 U of DV works nicely.

Optimum seems to be

2U of Taq + 0.05 or .02 or .01 U of Deep vent. But increasing the DV to .1U gives cleaner product.

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Witnessed & Understood by me, [signature] Date 1/4/95

Invented by

Recorded by K. Sitaraman Date 12/28/94